Progress Report #1
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Title: ***Fish Otolith and Condition Study***

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This component of the POD work-plan focuses on obtaining growth-related and rearing location information for delta smelt and striped bass from otolith microstructure and microchemistry analyses. The overall goal is to measure cohort-specific growth and condition for these species to determine the degree of growth-related impairment due to declines in pelagic food resources or exposure to toxic chemicals. In addition, otolith microchemistry is being used to help identify regions of the estuary that contribute more or less to annual year-class success.

Here, we report on progress to analyze temporal and spatial information from otoliths of delta smelt collected in the 20mm post-larval survey , mid-water trawl survey, and spring Kodiak trawl survey in 1999 and 2004. The objective is to compare birthdates and growth-rates of post-larvae, and natal history of adult fish, between a year of reasonably good year year-class success (1999) and a year of record low abundance (2004).

METHODS

Fish samples
Larval and post-larval delta smelt were collected from May through June of 1999 at six locations: Napa River (345, 346), Lower Sacramento River near Cache Slough (716), the Central Delta near the Mokelumne River (902), the South Delta near the San Joaquin River (915) and, the confluence of the Sacramento and San Joaquin River near Sherman Island (703,809) and Montezuma Slough (609) . These areas were chosen as possible natal areas based on the occurrence of yolk-sac larvae at only these sites during the 20-mm Survey (California Department of Fish and Game 2004). Subsequently, in November of 1999, we collected adult delta smelt during the CDFG mid-water trawl survey. In 2004 pre-spawned delta smelt were collected during the Spring Kodiak Trawl Survey. The collection sites represent the extent of the adult spatial distribution (www://delta.dfg.ca.gov/midwatertrawl.html) (Figure 1). All fish were preserved in 95% ethanol in the field and returned to the lab.

Fish analyses
In the laboratory, fish were measured for standard length, with no correction for shrinkage. The sagittal otoliths were removed, weighed on a microbalance and cleaned in Milli-Q water. Otoliths were then mounted on glass slide, affixed with acrylic glue and polished on one side with 3-micron lapping film to expose the core. Lastly, the otoliths were washed with 1% nitric acid for 5 to 10 seconds, rinsed in an ultrasonic waterbath for 5 minutes, and dried under a class 100 laminar flow hood. For age and measurements of individual growth

increments, otoliths were then polished and examined under a light microscope at 400-1000x magnification using computer-assisted image analysis (see Hobbs et al. *In revision*-b for detailed methods)

Trace elemental concentrations were analyzed with laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS) (Agilent Technologies 7200A ICP-MS, coupled with a Nd:Yag, New Wave Technologies Laser). Three replicate spots at the core of each otolith was quantified at a spot size of 20μm, with the laser operating at 5 Hz, and a scan rate of 30 seconds. Initially we examined 18 elements in larval and adult delta smelt otoliths (Li, Be, B, Na, Mg, Al, S, Ca, Mn, Mg, Fe, Se, Sr, Ba, W, Hg, Pb, U). Three elements were consistently detectible above background levels in both larval and adult otoliths (Sr, Mg, Ba). All element concentrations were standardized with NIST 612 glass standards (National Institute of Standards and Technology- U.S. Department of Commerce.). The stability of laser conditions was evaluated by examining the relative standard deviation (RSD) of target elements in the NIST 612 glass standards. Estimates of precision (%RSD) based on repeated measures of NIST 612 glass standards (30 analyses) were as follows: Na/Ca (4.0%), Mg/Ca (7.6%) Sr/Ca (6.5%) and Ba/Ca (10.6%). All other elements were eliminated from statistical analysis as precision estimates were less than 20%.

RESULTS & DISCUSSION

Otolith microstructure

The spawning season for delta smelt was longer in 1999 than in 2004. Spawning season, as indexed by water temperatures of 15-20 ^{o}C (Bennett 2005), occurred from April 16-June 18 (63 days) in 1999 and from March 13-May 29 (47 days) in 2004 as shown by spring water temperatures averaged over Suisun Bay and Delta (Figure 1). Back-calculated birthdates of fish fall within this period in 1999, and begin one week prior to 15 ^{o}C in 2004. Given that our back-calculation estimates are accurate to about 5 days, and that an overall measure of water temperature does not reflect finer scale variability in the delta smelt spawning habitat, the back-calculated birth-dates are very consistent with the water temperature estimates of the duration of each spawning season.

Length of the spawning season is one of the few environmental variables significantly associated with delta smelt recruitment success, as indexed by the fall mid-trawl (pre-adult) abundance index (Bennett 2005). Including data from 2003-2004 does not change this relationship. In 1999, the spawning season was about 2 weeks longer than in 2004 possibly contributing to higher abundance of delta smelt in 1999. However, the values for 2003 and 2004 fall much lower than expected considering the overall relationship (Figure 2). This suggests that a relatively narrow spawning season (47 days) was not the only factor contributing to the record low abundance of fish in 2004.

Overall, fish lengths and ages are similar between years, although the 2004 samples consist of some older/larger fish (Figure 3). Average growth rate is slightly faster in 1999 than 2004. Linear regressions fitted to length at age plots show that average growth rate (slope) was 0.37mmd^{-1} (S.E. = 0.017, r = 0.90, P < 0.0001) in 1999, and was 0.34mmd^{-1} (S.E. = 0.026, r = 0.82, P < 0.0001 in 2004 (Figure 3). Small changes in average growth rate can have significant influences on size structure and year-class success in the future. To explore this issue, size distributions for each year were projected from the age at capture to 100 days of age using average growth rates. The projection end-point (100 days) reflects the age at which overall delta smelt growth rates begin to asymptote (see Bennett 2005). Some fish were slightly larger in 2004 than 1999 (Figure 3, 4). However, projected size distributions become similar between years at 100 days, suggesting the slightly higher average growth in 1999 may have contributed to higher year-class success (Figure 4).

To examine finer-scale variation in growth between years and among sampling locations, we standardized the overall growth rate-age relationship. To do this, exploratory analysis showed the relationship was best-fit using a negative exponential model (Figure 5). Residuals from this relationship are positively associated with a simple measure of fish condition (K= standard length^3/weight) for the 2004 samples, indicating fish in better condition had higher growth-rates. Fish weights are not available for the 1999 samples, however, relationships using fish length are similar to those using condition for 2004 (Figure 6); this is not surprising because fish length obviously dominants the condition index, K. Thus, fish length was used as a surrogate for fish condition in comparisons between 1999 and 2004.

Residual otolith growth and fish length are positively associated in 1999 and 2004 (Figure 6, 7). For 1999, the relationship was best-fit using a least-squares regression ($r = 0.43$, $P < 0.0001$), and by using a robust regression which reduced the influence of outlying values in 2004 ($t = 3.78$, $P = 0.006$, Figure 7). In 1999, fish collected from the Napa River (Stations 345-346) and Sacramento River near the Cache Slough complex (Station 716) were in better condition (larger) and higher residual otolith growth rates (62% and 67%, Figure 7). Similarly, in 2004, 81% of fish collected at Station 716 also had positive residual growth and were in better condition. In each year, fish from the San Joaquin River and south Delta (Stations, 809, 901-915) and near Sherman Island (Station 703) had the poorest growth and condition (Figure 7). In 1999, 66% of fish examined from the south Delta and 78% of fish from Station 809 had negative residual growth and were smaller; and in 2004, 50% of the fish from Station 809 and 61% from Station 703 had poorer growth and condition (Figure 7). Interpretations of these results from other locations, such as Suisun Marsh, are heavily influenced by small sample sizes.

Otolith Microchemistry

Significant spatial variability in otolith trace elements was detected in the larval delta smelt reflecting different larval origins. A difference in trace elemental chemistry among the five hypothesized natal sites suggests the existence of distinct geographic cohorts within the population. (MANOVA: $df = 20,84$; $F = 1.436$; $p = 0.0036$) (Figure 8A,B). The samples collected in the Napa River had higher Mg concentrations relative to other locations. Fish from the Napa River were readily distinguishable from all other locations on the CV1, Mg/Ca (Figure 8A,B), while fish from the Sacramento River were negatively associated with the second canonical variate (CV2) and lowest in Sr/Ca (Figure 8A,B). Suisun Bay was readily distinguishable along CV2 having higher Sr/Ca concentrations.

Linear Discriminant Function Analysis (LDFA) determined the accuracy of classification to natal origin, with success rates evaluated using jackknife and cross validation techniques. Successful classification ranged from 25% for fish collected from the confluence to 90% for those collected from the Napa River. However, the overall success rate for jackknife classification for larval fish was only 51%. Since overall classification of larval fish to their natal origins was low, and discrimination among sites within the Delta region was poor, we combined these four sites: Sacramento River (ND), Confluence (C), central Delta (CD), and the south Delta (SD) into a singe class: Delta (D). This increased classification success rates for the Delta (90%) Napa sites (86%) and Suisun sites (74%).

Adult fish from 1999 were then classified to a natal region using Fisher discriminate function coefficients from larval LDFA with Delta sites combined. LDFA was used to evaluate accuracy of our classifications. The success rate was high, with (95%) of the fish classified back to the Napa River and 100% of fish classified back to the Delta. When, the percent contribution for each natal area was compiled, a majority of the adult fish (74%) originated in the Delta, and 26% were derived from the Napa River (Figure 8A,B). Pre-spawning adults from 2004 were derived primarily of the Delta stock, because no fish from the Napa River were identified using trace element otoliths chemistry (Figure 8C,D).

These data clearly demonstrate the utility of otolith chemistry in determining the cohort structure of the delta smelt population. For example the 1999 year class, which was the 3rd largest in the last 20 years was derived of at least three cohorts; Delta, Suisun, and Napa River. Previously we identified the Delta cohort of the 1999 year class to consist of fish originating in the lower Sacramento River (716) and the Central-South Delta using otolith strontium isotope ratios (Hobbs et al 2005). Meanwhile, the 2004 year class did not contain a cohort from the Napa River and very few if any from a Suisun cohort. The population consisted of Delta and possibly Sacramento River fish (Figure 8C,D). These results suggest that the broader distribution of delta smelt may have contributed to higher year-class success in 1999.

Implications

Preliminary results of otolith microstructure and microchemistry suggest that higher year class success in 1999 may be attributed to a relatively long spawning season, and a broader distribution into habitats which facilitated higher cohort growth rates. In 1999 the population consisted of cohorts from the Delta, Suisun Marsh, and Napa River, whereas only a Delta cohort was identified in 2004. In 1999, post-larval delta smelt had higher average growth rates than in 2004; and, regional comparisons indicate that fish rearing in the Napa River and upper Sacramento River had the highest growth rates. Similarly, fish rearing in the upper Sacramento River had the highest growth rates in 2004. Consistently, fish rearing in the San Joaquin and south Delta regions had the lowest growth rates and condition in 1999 and 2004. These results are also consistent with those from investigations in the low-salinity zone in 1996 in which delta smelt from northern Suisun Bay had higher feeding success and were in better condition than those collected from southern Suisun Bay near the lower the San Joaquin River (Hobbs et al., in review). The implications of these results for understanding differences in year-class success between years are contingent on analyses of fish at life stages between the post-larval and pre-spawning periods, as well as synthesis with histopathological results from Dr. Swee Teh.

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FIGURE LEGENDS

Figure 1. Trends in spring water temperatures during 1999, 2004, 2005, and estimated spawning seasons for delta smelt (15-20^{o}C), as well as birth-date distributions of fish from the 20mm post-larval survey in 1999 and 2004.

Figure 2. Relationship between the fall pre-adult (MWT) index and the estimated durations of spring spawning season. Numbers are years.

Figure 3. Length at age relationships for post-larval delta smelt in 1999 and 2004. Numbers are sampling stations in the 20mm survey.

Figure 4. Projections of size at catch distributions to 100 days using average growth rates for post-larval delta smelt in 1999 and 2004.

Figure 5. Overall relationship between growth rate and age for post-larval delta smelt combining data for 1999 and 2004. Fitted line is a declining exponential curve.

Figure 6. Relationships between residual growth rate and fish length (1999 & 2004) and fish condition (2004).

Figure 7. Relationships between residual growth rate and fish length for 1999 and 2004, showing the percent of fish from different locations with negative residual growth. Numbers are sampling stations.

Figure 8. **A**-plot of Sr/Ca and Mg/Ca. Natal signatures (■) depicted by means of 10 larval –stage fish per site (20mm Survery CDFG) and error bars are 95% confidence limits. Adult fish (●) natal signatures (FMWT CDFG) are plotted on the same figure for direct comparison. **B**-plot of Ba/Ca and Mg/Ca of 1999 year class.
C-plot of Sr/Ca and Mg/Ca for 2004 yearclass. Colored elipses represent the 95% confidence interval of larval natal signatures from 1999 20mm Survey samples (A,B).
D- plot of Ba/Ca and Mg/Ca for 2004 yearclass. Adult samples were collected during the SKDT Survey CDFG. Sites are N, Napa River, SM Suisun Marsh, CD Central Delta, SD South Delta, WD West Delta, ND North Delta-Sacramento River.

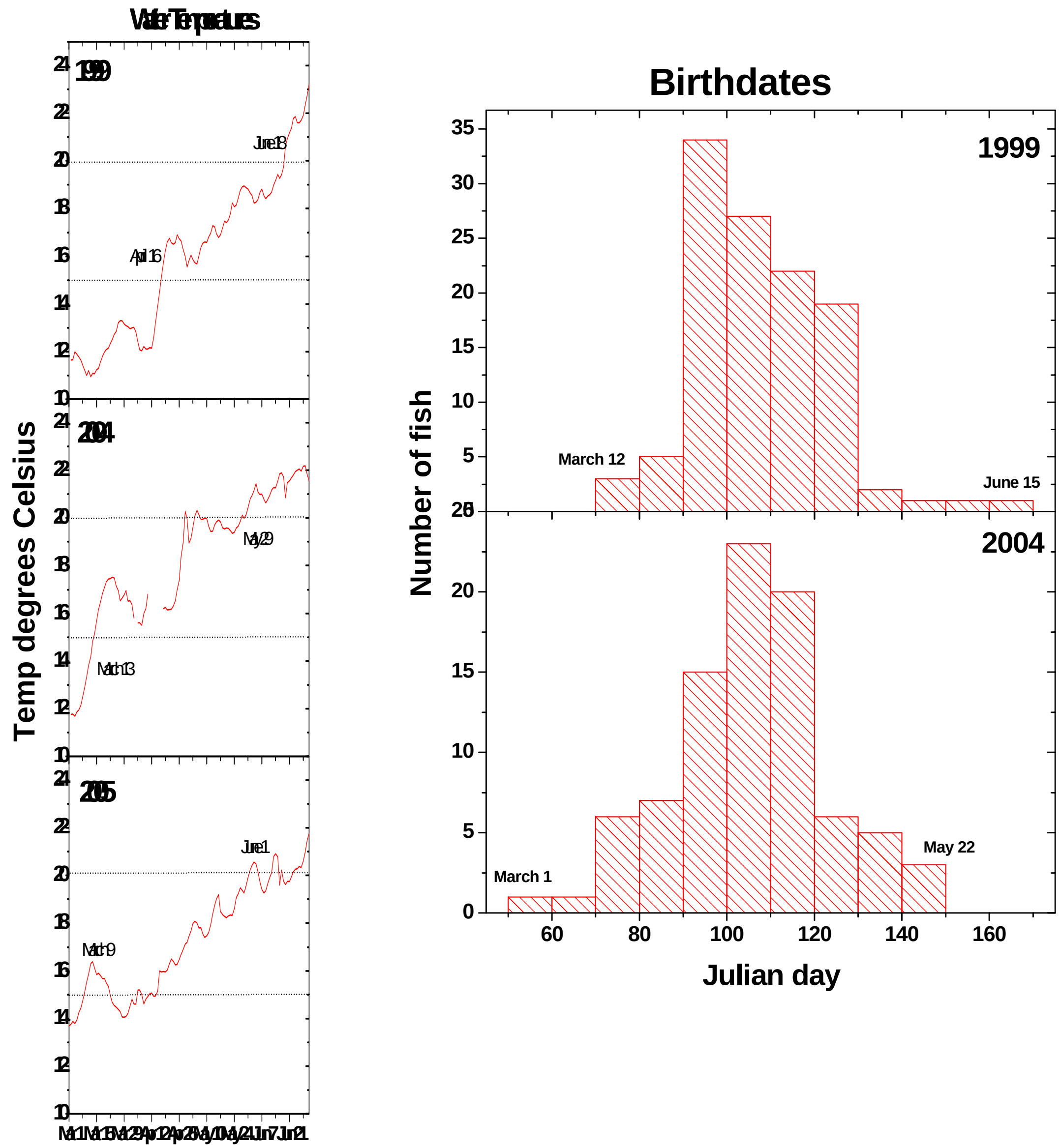

Figure 1.

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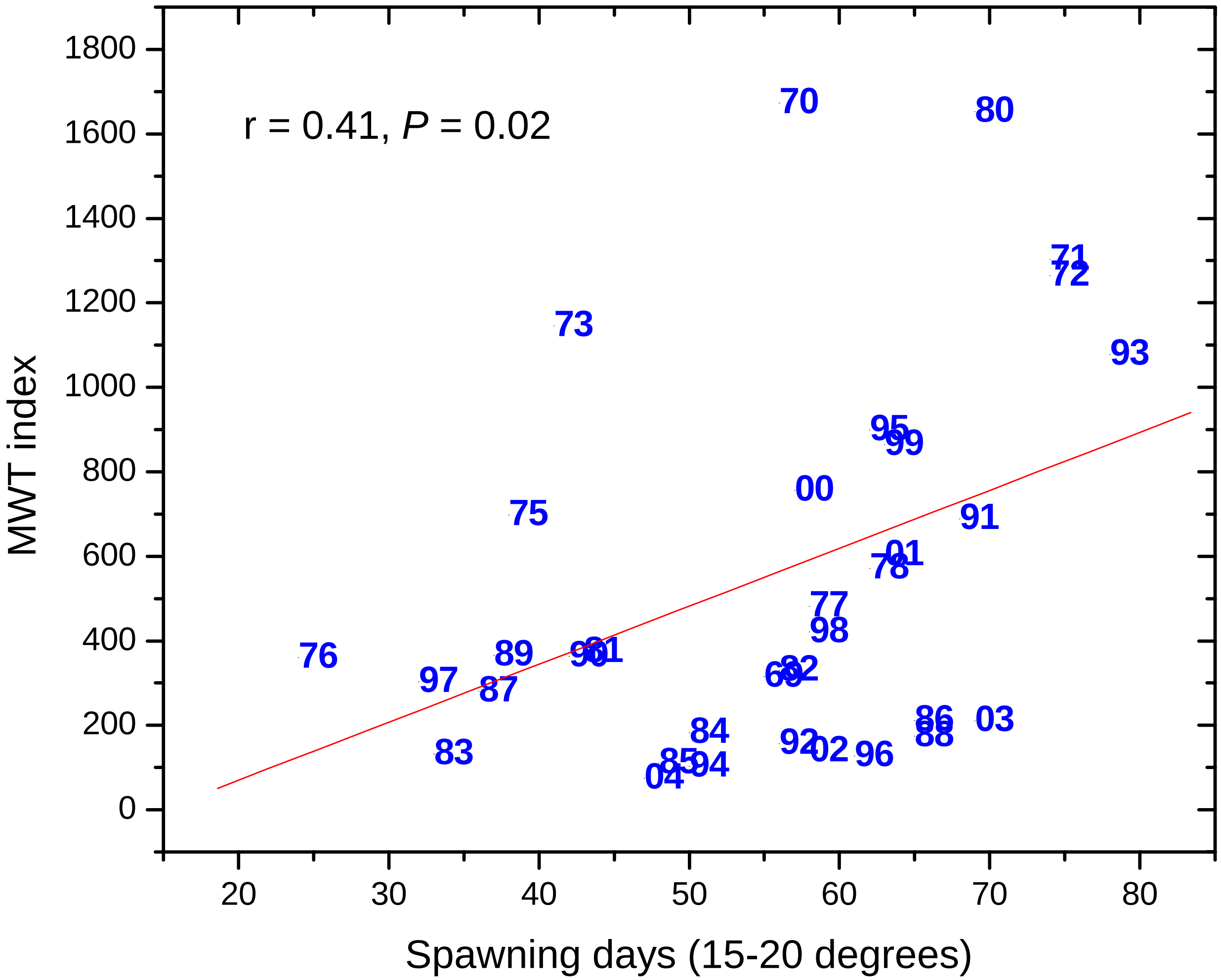

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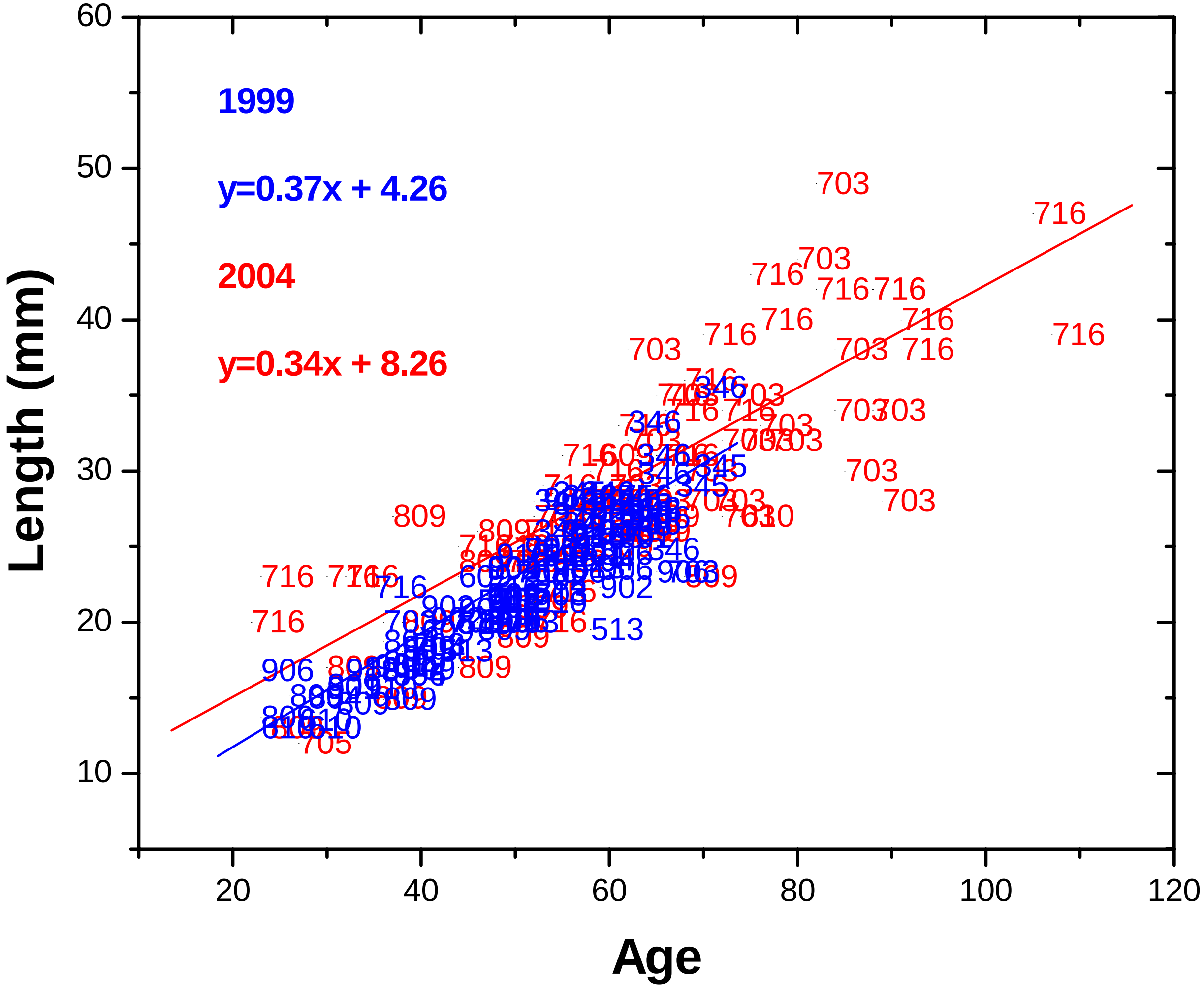

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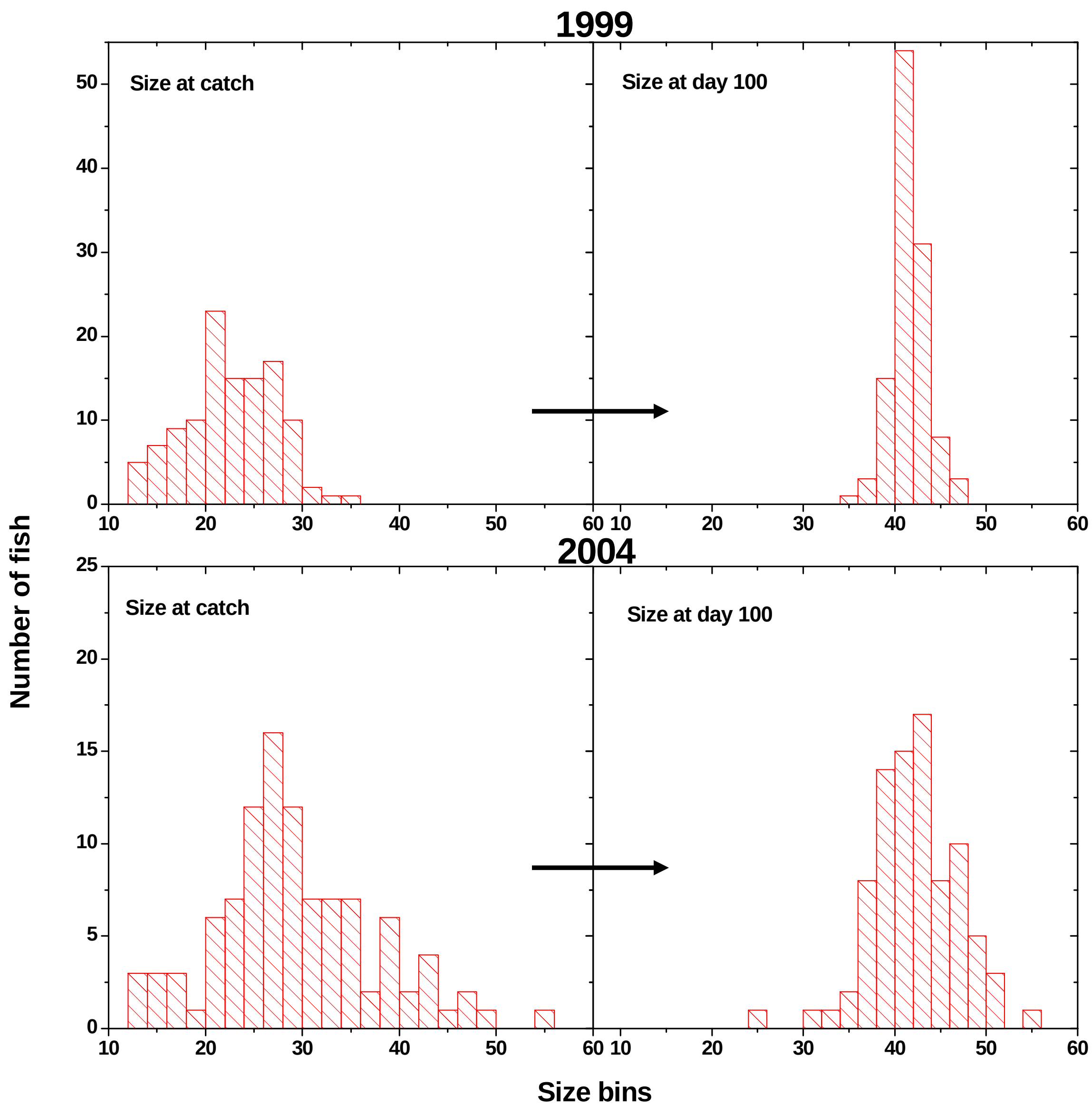

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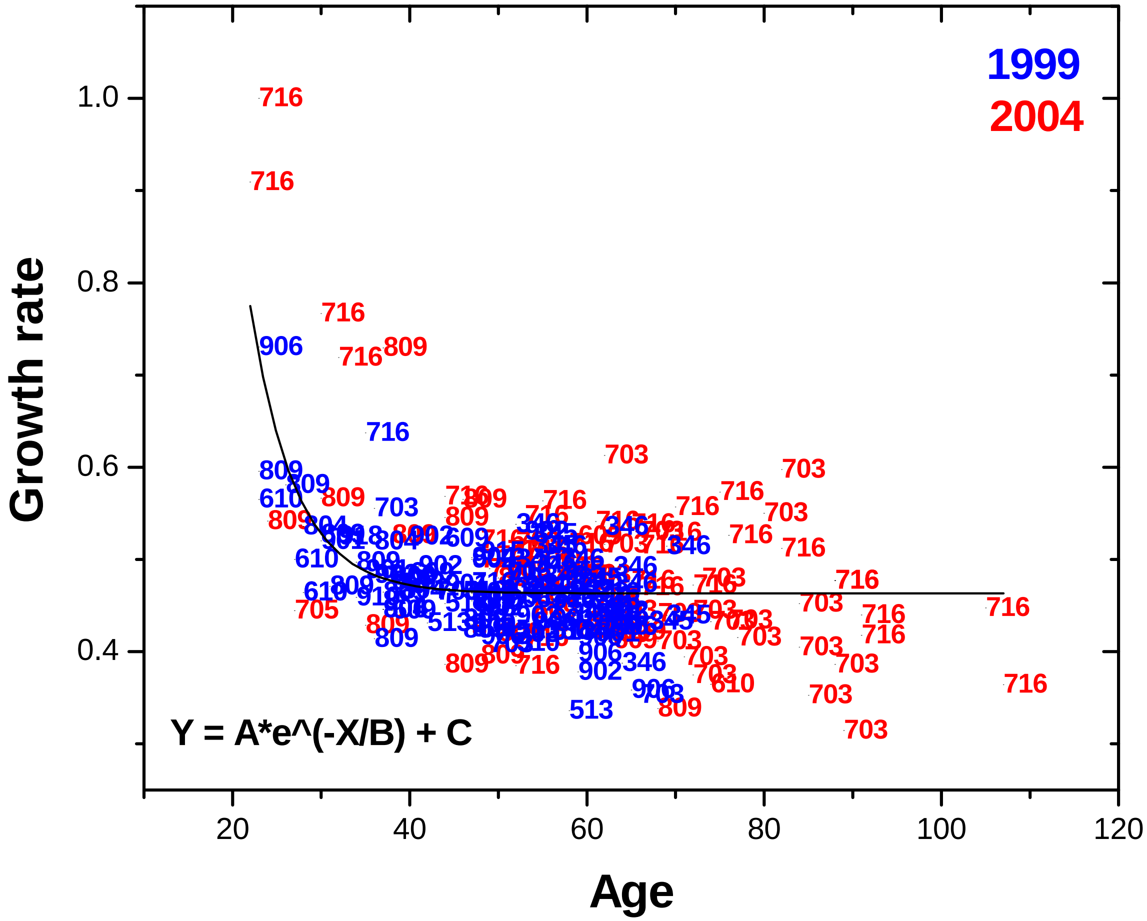

Figure 5.

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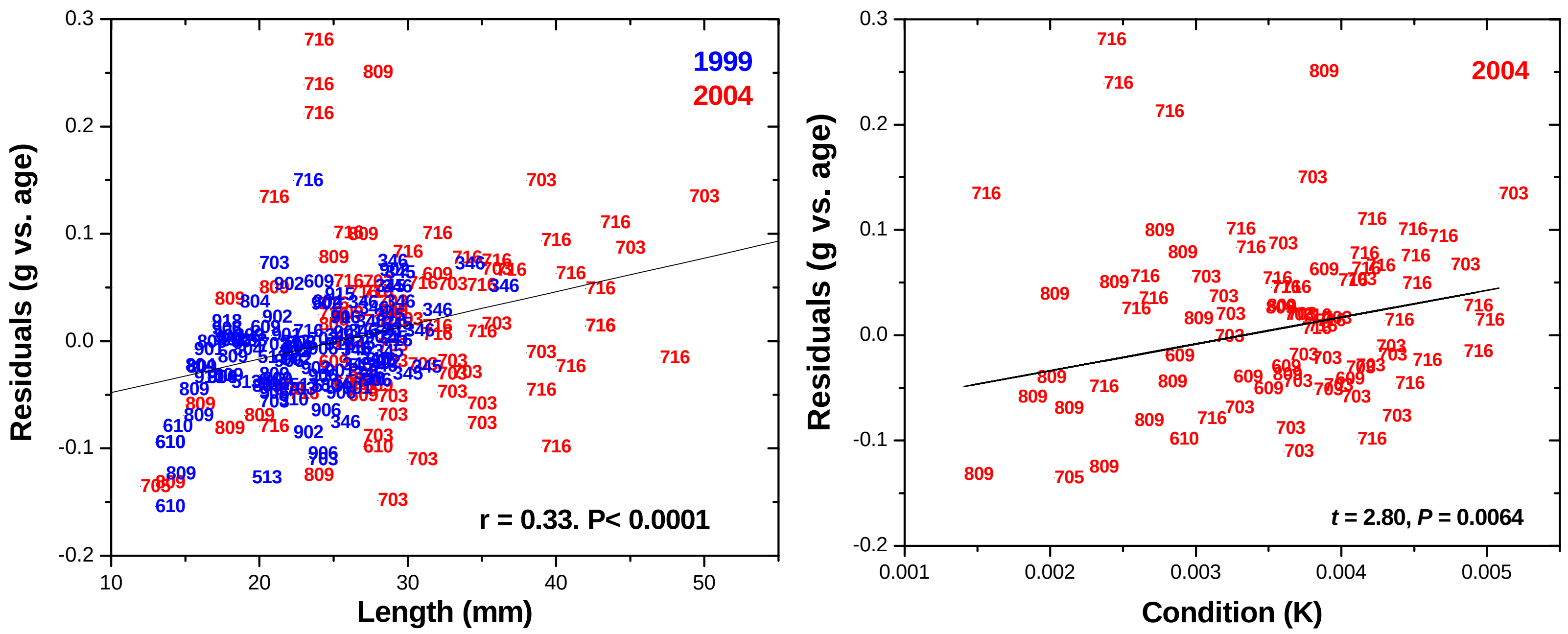

Figure 6.

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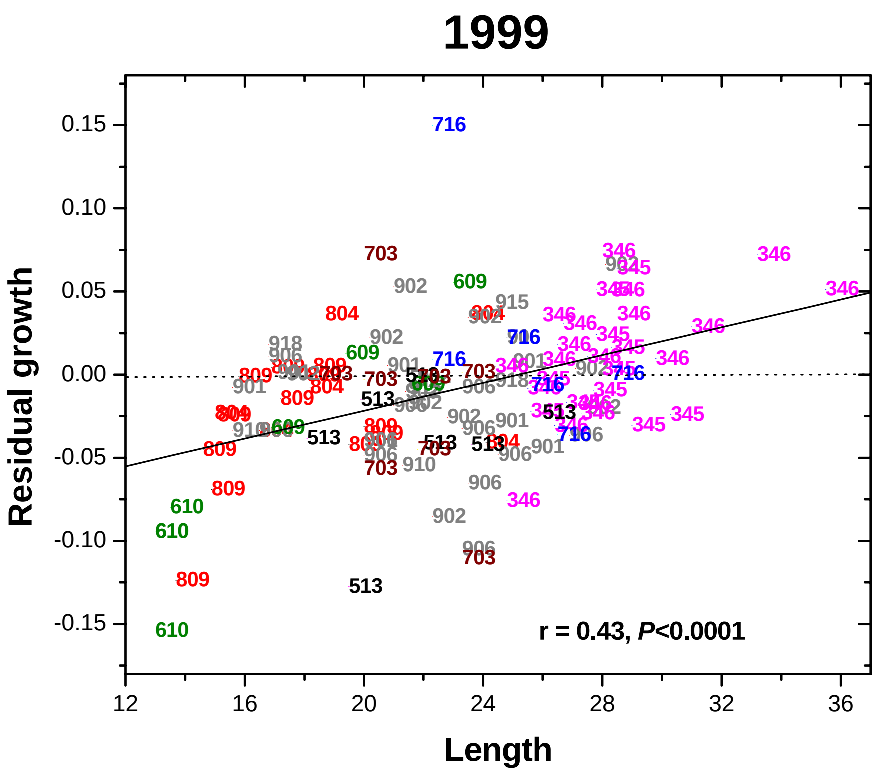

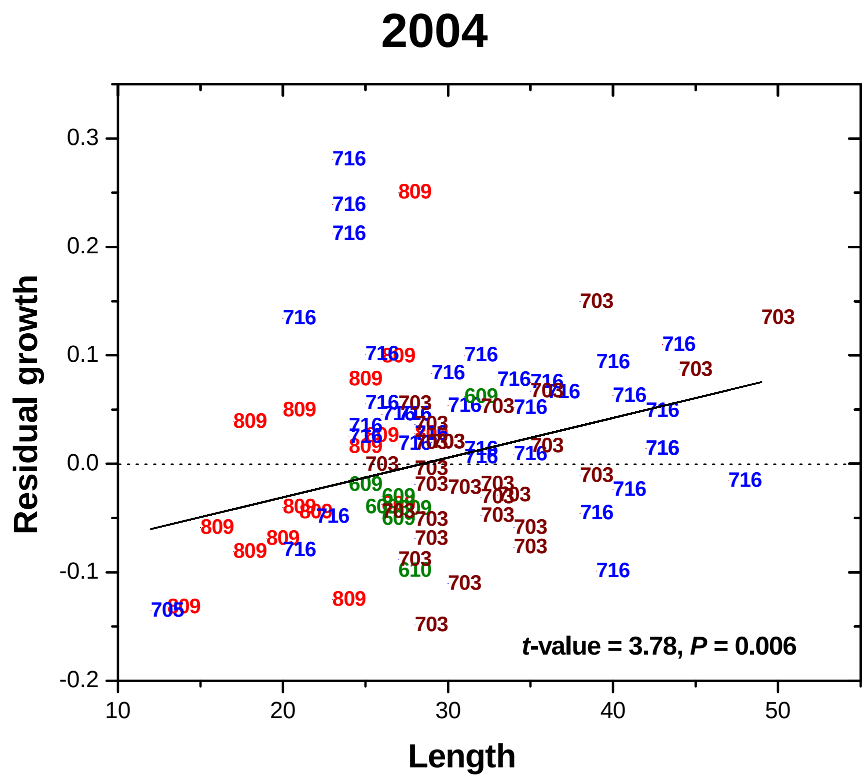

Location	N	% - residual gr.
S. J. River	18	78
S. Delta	38	66
Prospect Sl.	6	33
Suisun M.	9	78
Sherman Isl.	8	62
Suisun Bay	7	100
Napa R.	29	38

Location	N	% - residual gr.
S. J. River	16	50
Prospect Sl.	36	19
Suisun M.	7	86
Sherman Isl.	28	61

Figure 7.

This is a draft work in progress subject to review and revision as information becomes available.

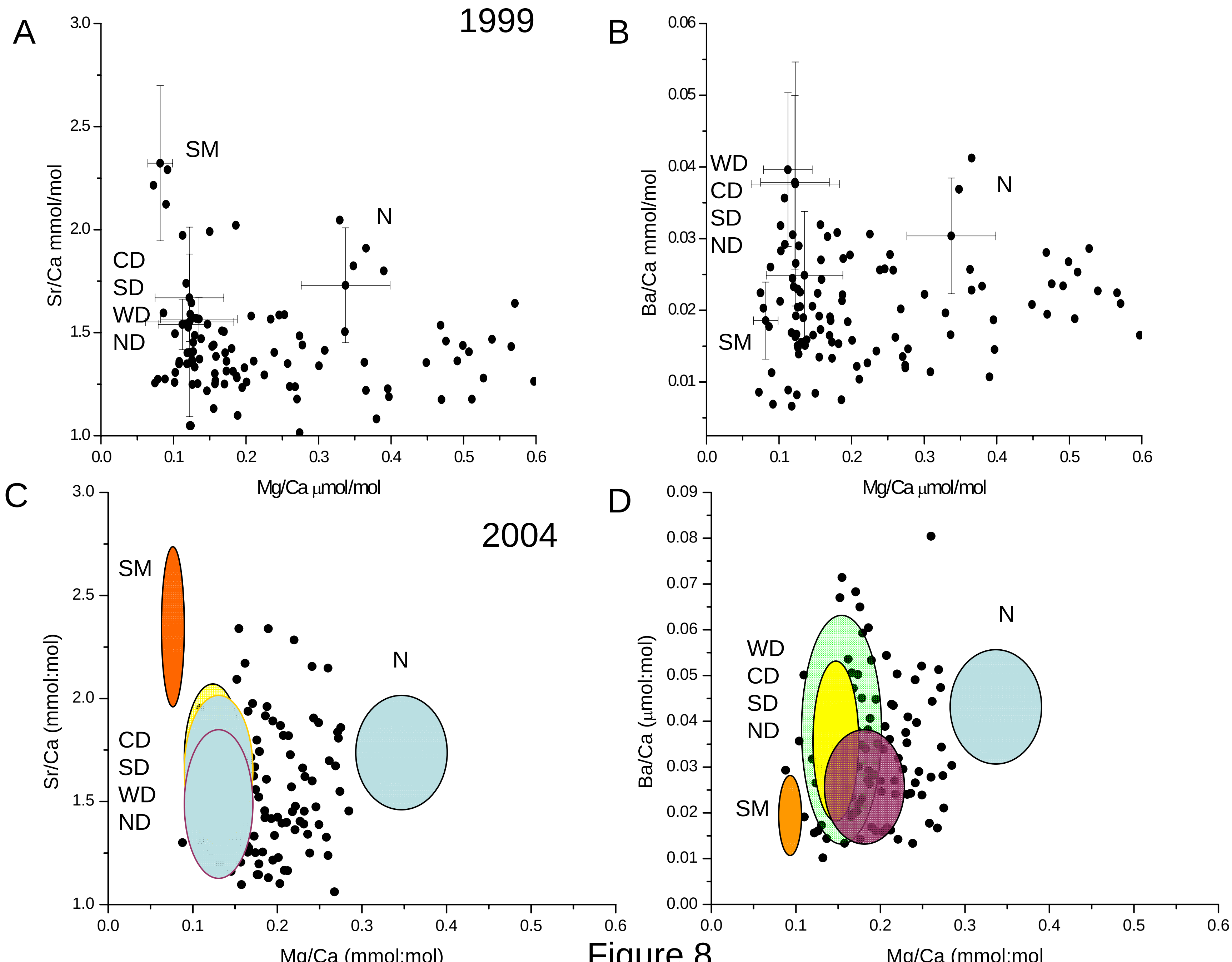

Figure 8.

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